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Toxicity of Chlorine to Juvenile Spot Leiostomus Xanthurus

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16. ABSTRACT

The sensitivity of juvenile spot, Leiostomus xanthurus, to total residual chlorine (TRC) in flowing sea-water was investigated. Incipient LC_{50} bioassays, histopathology, avoidance tests and the combined effect of thermal stress and TRC were used to assess sensitivity.

Estimated incipient LC_{50} values were 0.12 $mg l^{-1}$ TRC at 10°C and 0.06 $mg l^{-1}$ TRC at 15°C. Histological examination of spot used in the incipient LC_{50} bioassay at 15°C and sacrificed while alive indicated pseudobranch and gill damage occurred in individuals exposed to a measured TRC concentration of 1.57 $mg l^{-1}$. Spot exposed to lower concentrations of TRC, 0.02-0.06 $mg l^{-1}$ at 15°C and sacrificed alive showed no consistent tissue damage.

Simultaneous exposure of spot to thermal stress (5, 10 or 13°C above the acclimation temperature of 15°C) at measured TRC concentrations of 0.05-0.07 and 0.34-0.52 $mg l^{-1}$ demonstrated a significant, (X^2 with Yates correction, $P < 0.05$) increase in sensitivity to TRC with increased temperature and exposure times for some of the groups tested.

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TOXICITY OF CHLORINE TO JUVENILE SPOT, *LEIOSTOMUS XANTHURUS*

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Abstract—The sensitivity of juvenile spot, *Leiostomus xanthurus*, to total residual chlorine (TRC) in flowing sea-water was investigated. Incipient LC_{50} bioassays, histopathology, avoidance tests and the combined effect of thermal stress and TRC were used to assess sensitivity.

Estimated incipient LC_{50} values were 0.12 mg l^{-1} TRC at 10°C and 0.06 mg l^{-1} TRC at 15°C. Histological examination of spot used in the incipient LC_{50} bioassay at 15°C and sacrificed while alive indicated pseudobranch and gill damage occurred in individuals exposed to a measured TRC concentration of 1.57 mg l^{-1}. Spot exposed to lower concentrations of TRC, 0.02–0.06 mg l^{-1} at 15°C and sacrificed alive showed no consistent tissue damage.

Spot demonstrated temperature dependent avoidance responses to TRC. At 10°C, a concentration of 0.18 mg l^{-1} was required for significant (χ^2; $P < 0.05$) avoidance; at 15 and 20°C, spot showed significant avoidance of TRC concentrations as low as 0.05 mg l^{-1}.

Simultaneous exposure of spot to thermal stress (5, 10 or 13°C above the acclimation temperature of 15°C) at measured TRC concentrations of 0.05–0.07 and 0.34–0.52 mg l^{-1} demonstrated a significant, (χ^2 with Yates correction, $P < 0.05$) increase in sensitivity to TRC with increased temperature and exposure times for some of the groups tested.

INTRODUCTION

Major sources of chlorine contamination in the marine environment are related to chlorination of secondary sewage treatment wastes with subsequent discharge into coastal and estuarine waters, and chlorination of seawater used for single-pass cooling of thermal electric generating plants (White, 1972, 1973; Markowski, 1959).

Few toxicological data are available on the effects of chlorine and chlorination products from sewage treatment on marine fishes. Tsai (1968, 1970) observed decreases in the occurrence and abundance of brackish water fishes in certain areas of the Upper and Little Patuxent Rivers, Maryland that received chlorinated sewage effluents.

Meldrim *et al.* (1974) determined behavioral and metabolic responses of several estuarine fishes and invertebrates to combinations of chlorine, temperature, salinity, dissolved oxygen, and light. Stober & Hanson (1974) demonstrated that juvenile chinook, *Onchorhynchus shawytscha*, and pink salmon, *O. gorbuscha*, become less tolerant to residual chlorine as sea-water temperature and exposure time increases. Hoss *et al.* (1975) also demonstrated an additive effect when both larval flounder, *Paralichthys* sp., and menhaden, *Brevoortia tyrannus*, were exposed simultaneously to thermal shock and chlorine.

Our study was conducted with juvenile spot, *Leiostomus xanthurus*, a fish which ranges from Texas to Massachusetts Bay (Bigelow & Schroeder, 1953). This species spawns offshore from October through February (Hildebrand & Schroeder, 1928; Pearson, 1929) and larvae generally begin to appear in low-salinity estuaries throughout the range in late December or early January (Gunter, 1938; Massman, 1954).

Because of its dependence on estuarine nursery areas, spot is one species likely to be affected by chlorinated sewage effluents (Virginia State Water Control Board, 1974) and entrainment in thermal electric generating stations (Hoss *et al.*, 1971). To determine the sensitivity of juvenile spot to total residual chlorine (TRC) in sea-water, we used incipient LC_{50} bioassays (Sprague, 1964, 1969), histopathology, avoidance tests, and the combined effect of thermal stress and TRC.

MATERIALS AND METHODS

Juvenile spot (total length 10–20 mm, wet weight 0.10–0.15 g) were collected during January and February 1975 from We Creek, a tidal tributary of the North Edisto River estuary, Wadmalaw Island, South Carolina. Collections were made just after dark on rising tides during moonless or overcast nights. A custom-made, 2.0 × 0.7 m, surface plankton net with 1.0 mm mesh was used (Lewis *et al.*, 1970). Sea-water temperatures ranged from 11.1–20.0°C and salinity from 19–25‰ during collections.

Spot were transferred to the laboratory and stocked in aerated rectangular flow-through tanks, 60 cm × 50 cm × 25 cm (vol. 80. 1), with a flow rate of 20–40 l h^{-1} of unfiltered sea-water. Fish were fed mysid shrimp, *Neomysis americana*, and *Artemia* sp. nauplii twice daily during laboratory holding and temperature acclimation. Dissolved oxygen during collection, temperature acclimation, and all tests with chlorine was greater than 6.0 mg l^{-1}. Sea-water pH ranged from 7.7–7.9 during this study.

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Temperature acclimation

Spot were acclimated in flowing sea-water to 3 temperatures, 10, 15 and 20 ± 1°C. Fish collected at low ambient water temperatures, 11.1–14.0°C, were acclimated to 10 or 15°C for a minimum of 5 days; those collected at ambient temperatures of 18.0–20.0°C were acclimated to 20°C for at least 4 days (temperature change during acclimation did not exceed 1.0°C day^{-1}). A 2000-watt quartz immersion heater and Frigid* titanium coil water chiller maintained acclimation temperatures. Ambient salinities ranged from 18–24‰ during temperature acclimation.

Incipient LC_{50} bioassays

To determine the incipient LC_{50}, 16 temperature-acclimated juvenile spot were placed in each of 7 acrylic plastic flow-through aquaria, 27 cm × 15 cm × 8.7 cm (vol. 4, 1) that received 18 l h^{-1} of sea-water. After 24 h of acclimation in the treatment aquaria, chlorine (Mallinckrodt NaOCl, Analytical Reagent, minimum 5.0% NaOCl) was introduced by syringe pump to six of the aquaria. Perforated acrylic plastic baffles covered with 0.5 mm mesh nylon screen were located at the front and rear of each treatment aquarium. One baffle formed a chlorine 'mixing chamber' at the entrance point of sea-water and chlorine addition to the flow-through tank; the other prevented loss of juvenile spot through the standpipe drain. Bioassays were conducted at 10 and 15 ± 0.5°C. Salinities ranged between 20 and 24‰. Spot were fed *Artemia* nauplii twice daily during chlorine exposure. Observations for time to death were made continuously for the high exposure concentrations of chlorine and at 2–3 h intervals for low-exposures. Tests were terminated after 8 days. Survival of control animals was greater than 95% in each test. The daily photoperiod was 12 h light of 1700–2000 lux intensity from fluorescent 'cool white' lamps.

Histology

Surviving juvenile spot from the incipient LC_{50} bioassay conducted at 15°C were removed from their respective treatment and control aquaria and preserved in Davidson's Fixative. After 24 h, fish were transferred to 50% ethanol for 2 h and then to 70% ethanol for storage. Spot were dehydrated, infiltrated with paraffin and paraffin-embedded for sectioning sagittally and parasagittally at 7 μm. Sections were stained with Harris hematoxyline and eosin and mounted for light microscopy.

Avoidance tests

An avoidance apparatus (Fig. 1) similar to that of Sprague (1968) was used to determine the response of spot to TRC in flowing sea-water. Tests were conduced at 10, 15, and 20 ± 0.5°C. Salinities ranged from 19–22‰.

Our avoidance apparatus was constructed of clear acrylic plastic (Fig. 1). Eighteen 1 h^{-1} of sea-water was syphoned from the head trough into each mixing box. Sixteen spot were placed in the avoidance apparatus through the stoppered fish-entrance, chlorine-sampling ports (8 through each port) adjacent to the center interface drain. The flow of 18 l h^{-1} of sea-water into each side of the avoidance chamber (38.1 mm i.d.) produced a water velocity of approximately 0.375 cm s^{-1} in the direction of the interface drain. Spot oriented into this current and usually remained in the side of the chamber to which they were introduced. Overhead fluorescent 'cool white' lighting provided 2000 lux of uniform illumination over the avoidance apparatus. Nylon plankton netting (250 μm mesh) was placed over each end of the avoidance chamber to prevent passage of juvenile spot into the mixing boxes. Immediately after placing fish in the chamber, a black plastic shield was erected around the apparatus to prevent outside disturbance.

Spot were acclimated in the apparatus for 30 min. Five counts of the number of fish on each side of the interface drain were then made at 5-min intervals. Immediately after making the fifth count, chlorine was added by syringe pump to one mixing box. A second series of five counts was then made at 5-min intervals to determine the number of fish on each side of the interface drain. Total residual chlorine concentrations were measured immediately after each avoidance test. Water samples were taken from sample tubes in the fish entrance-chlorine-sampling ports.

The avoidance apparatus was emptied and repeatedly flushed with large volumes of sea-water for 15–20 min after each test. Each test was then repeated (using new fish) with chlorine added to the alternate end of the avoidance apparatus.

The number of fish observed on respective sides of the interface drain for each of the five pre-chlorine exposure (control) intervals was subjected to individual chi-square analysis (assuming a 1:1 ratio for the two classes) and the individual χ^2 values summed. A pooled chi-square analysis was also conducted on the sum of the fish observed on each side of the interface. The χ^2 values for each of these tests were then compared for heterogeneity (Snedecor, 1956; Sokal & Rohlf, 1969). Similar analyses were conducted for the second series of five counts made after chlorine infusion had begun.

The analyses were designed to determine (1) existence of differences in the observed and expected ratios for the sums of individual and pooled observations prior to addition of chlorine, (2) differences in observed and expected ratios for the sum of individual and pooled observations after addition of chlorine had begun, and (3) heterogeneity between the sum of the individual observations and the pooled observations prior to and after addition of chlorine.

TRC-temperature interaction

Since estuarine fish subjected to residual chlorine in power plant effluents are likely to simultaneously experience rapidly increased water temperatures, we conducted tests to determine the combined effects of these factors. A matrix design, after Stober & Hanson (1974), was selected. We used two TRC ranges (0.05–0.07 and 0.34–0.52 mg l^{-1}), four temperatures (15°C–the acclimation temperature; 20, 25 and 28°C), and four exposure times (7.5, 15, 30 and 60 min). Twelve spot were exposed to each TRC-temperature-time regime in flowing-sea-water. After exposure, they were placed in a flowing-sea-water aquarium at 15 ± 0.5°C for 24 h and observed for number of survivors.

Spot were fed *Artemia* nauplii two hours before each test. Small numbers of *Artemia* were also added to the recovery aquarium during the 24 h depuration interval. Total residual chlorine concentrations in the exposure tanks were determined before and after each test.

Chi-square analysis (2 × 2 contingency table with Yates correction, $P < 0.05$) was used to compare the response of control and experimental spot at each TRC-temperature-time interval.

Chemical analysis

Total residual chlorine (TRC) concentrations (free + combined chlorine and other chlorine produced oxidants) were measured with a Wallace and Tiernan amperometric titrator. Phenylarsine oxide and pH buffers used were standard Wallace and Tiernan reagents. The 5% KI solution and NaOCl (Mallinckrodt, Analytical Reagent, minimum 5.0% NaOCl stock solutions were prepared in chlorine demand-free water.

Stock solutions for all biological tests and chemical analyses were kept in brown glass bottles at 4.0°C prior to use.

* ®Mention of trade names does not imply endorsement by the U.S. Environmental Protection Agency.

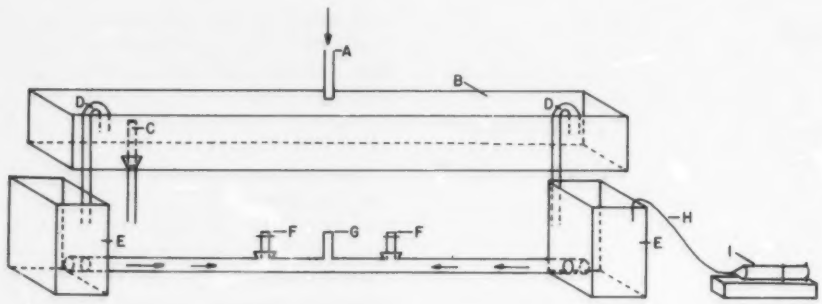


Fig. 1. Avoidance apparatus used for determining the response of juvenile spot to TRC in flowing seawater. *A*. Temperature controlled seawater supply, *B*. Head trough, *C*. Standpipe overflow for maintaining constant head, *D*. Calibrated 18 l h^{-1} syphons, *E*. Sea-water–chlorine mixing compartments, *F*. Fish-entrance chlorine-sampling ports, *G*. Interface-drain, *H*. Chlorine delivery tube, *I*. Syringe pump. → Direction of water flow.

Table 1. Nominal concentrations of chlorine added to flowing sea-water as NaOCl and total residual chlorine (TRC) measured at indicated test temperatures

Nominal NaOCl concentration added, mg l^{-1}	Measured TRC, mg l^{-1} Test temperature °C 10	15
5.6	2.48	1.57
3.2	0.85	0.60
1.8	0.27	0.22
1.6	0.22	0.17
1.4	0.18	0.14
1.0	0.12	0.08
0.56	0.04	0.04
0.32	0.02	0.02

Total residual chlorine determinations were conducted by procedures outlined in Standard Methods (APHA, 1971). Particular emphasis was given to sample handling and time required to perform titrations. All samples were taken directly from the treatment aquaria or avoidance apparatus and titrated to an end point within two min. Agitation of samples was minimized to reduce potential error caused by flashing-off or photodecomposition of residual chlorine (EPA, 1974). Table 1 summarizes the nominal concentrations added and measured total residual chlorine concentrations during the incipient LC_{50} bioassays at 10 and 15 °C. Nominal concentrations of NaOCl added the TRC levels measured are typical or those in other tests (including avoidance and temperature stress-chlorine regimes) in this study.

RESULTS

Incipient LC_{50} Bioassays

The incipient LC_{50} was estimated by determining the portion of the toxicity curve asymptotic to the *Y* axis and reading the corresponding TRC concentration from the *X* axis below (Fig. 2).

Regression analysis indicated that both toxicity curves fit the power equation ($Y = AX^b$). The correlation coefficient (r) for the 10°C curve was 0.91 and for 15°C, 0.87.

The time to 50 per cent mortality for fish exposed to chlorine at 10°C was slightly longer than that for those exposed to similar concentrations of chlorine at 15°C. The incipient LC_{50} at 10°C was 0.12 mg l^{-1} TRC and at 15°C, it was 0.06 mg l^{-1} TRC. No deaths were observed after 8 days for fish exposed to 0.04 and 0.02 mg l^{-1} TRC at either of the test temperatures.

Fish subjected to exposure concentrations of 0.22–2.48 mg l^{-1} TRC at 10 or 15°C showed a rapid increase in ventilation rate. The increase was evident in both opercular and mouth movement and greater lifting of the hypomandibular, operculum and mouth. These fish usually swam disorientedly at the surface of the treatment aquaria for a brief interval before dying. At high exposure concentrations, the time from death of the first fish until 50% mortality occurred was 30 min or less.

Fish exposed to TRC levels ranging from 0.08–0.18 mg l^{-1} usually showed a gradual increase in ventilation rate and accumulation of copious quantities of mucus along the posterior edge of the operculum. Small air bubbles (probably trapped in the mucus as fish gulped air at the surface) were often observed in fish that succumbed at these TRC concentrations.

Fish surviving exposure to 0.02 and 0.04 mg l^{-1} TRC for 8 days showed no obvious changes in ventilation rates or in swimming behavior at 10 and 15°C. However, we observed an apparent decrease in the intensity of the feeding response when *Artemia* sp. were added to tanks with a TRC level of 0.04 mg l^{-1} at both temperatures. This apparent decrease was not observed at a TRC level of 0.02 mg l^{-1} or for controls.

Pathology

Organ systems or tissues examined were brain, membranous labyrinth, gills, pseudobranch, liver, gut, pancreas, kidney, muscle and tegument. No pathological differences that were consistent and trend-reflecting were found between control fish and those exposed to measured TRC concentrations up to 0.60 mg l^{-1} at 15°C. However, fish exposed to 1.57 mg l^{-1} TRC consistently had alterations in

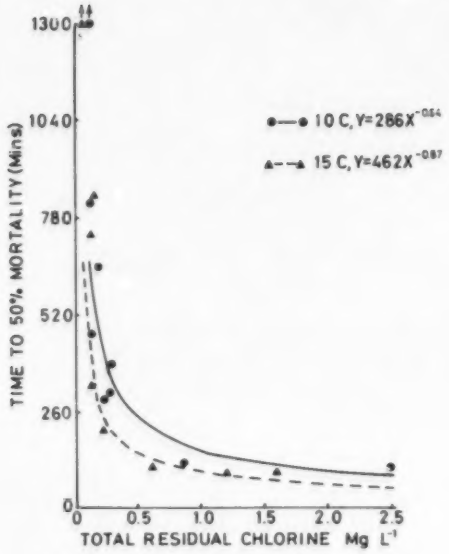


Fig. 2. Results of incipient LC_{50} bioassays. The incipient LC_{50} was estimated by determining the portion of the toxicity curve asymptotic to the Y axis and reading the corresponding TRC concentration from the X axis below. Incipient LC_{50} – 0.12 mg l^{-1} for 10°C, and 0.06 mg l^{-1} for 15°C.

pseudobranch and gill structures that were probably indicative of damage related to the test exposure.

The pseudobranch of juvenile spot is in a small pit in the dorsal buccopharyngeal epithelium beneath the membranous labyrinth and posterior to the eye. The pseudobranch epithelium consists of plump, eosinophilic cells that surround lamellar capillaries (Figs. 3 and 4). The larger structure resembles a normal gill filament and associated lamellae, except for the staining qualities and sizes of the cellular elements of the epithelium.

In juvenile spot exposed to 1.57 mg l^{-1} TRC for 95 min and sacrificed while still alive, pseudobranch lamellar epithelial membranes were distorted and separated from other gill tissues. The edematous separation of epithelial membranes gives the pseudobranch a scalloped border (Figs. 5 and 6).

Gill filaments in control fish were within normal ranges of artifactual and structural variation for spot (Fig. 7). Gills of fish from the 1.57 mg l^{-1} exposure, however, had severe pathological changes (Fig. 8). Gill filament and lamellar organization was disrupted by severe edema. Complete separation of squamous respiratory epithelium and capillary tissues in the gill lamellae occurred.

Scalloping of membranes in the pseudobranch (Fig. 6) has not been reported as a toxic effect in fish. As a response to TRC, membrane alteration visible at the light-microscope level probably indicates damage to the diffusion-transport mechanism of epithelial tissues. Similar changes involving the squamous respiratory epithelium and membranes of the gills suggests that damage to the diffusion-transport mechanism of gill tissues occurred with exposure to 1.57 mg l^{-1} TRC.

Avoidance tests

The avoidance response of juvenile spot to total residual chlorine in flowing sea-water was temperature-dependent. In tests conducted at 10°C, a measured TRC concentration of 0.12 mg l^{-1} did not cause consistently significant ($P < 0.05$), reproducible avoidance responses, although a measured TRC level of 0.18 mg l^{-1} did. In replicate tests at 15 and 20°C, a TRC concentration of 0.05 mg l^{-1} caused significant avoidance responses (Table 2).

Differences in the sum and pooled χ^2 values were not significant in any of the tests. For controls, this shows nearly equal distribution of the spot in a 1:1 ratio on each side of the apparatus (with little movement across the interface) prior to addition of chlorine. For chlorine-exposed spot, lack of heterogeneity indicates that movements of spot were uniformly in one direction (away from the side to which chlorine was introduced).

TRC-temperature interactions

Tests conducted to determine the combined effects of TRC and thermal stress showed that juvenile spot were most sensitive to TRC at high temperatures.

In preliminary tests, we determined that juvenile spot could survive direct transfer from the acclimation temperature (15°C) to 28°C in the absence of TRC (controls), provided they were returned to the acclimation temperature after 60 min or less exposure to the elevated temperature (Fig. 9).

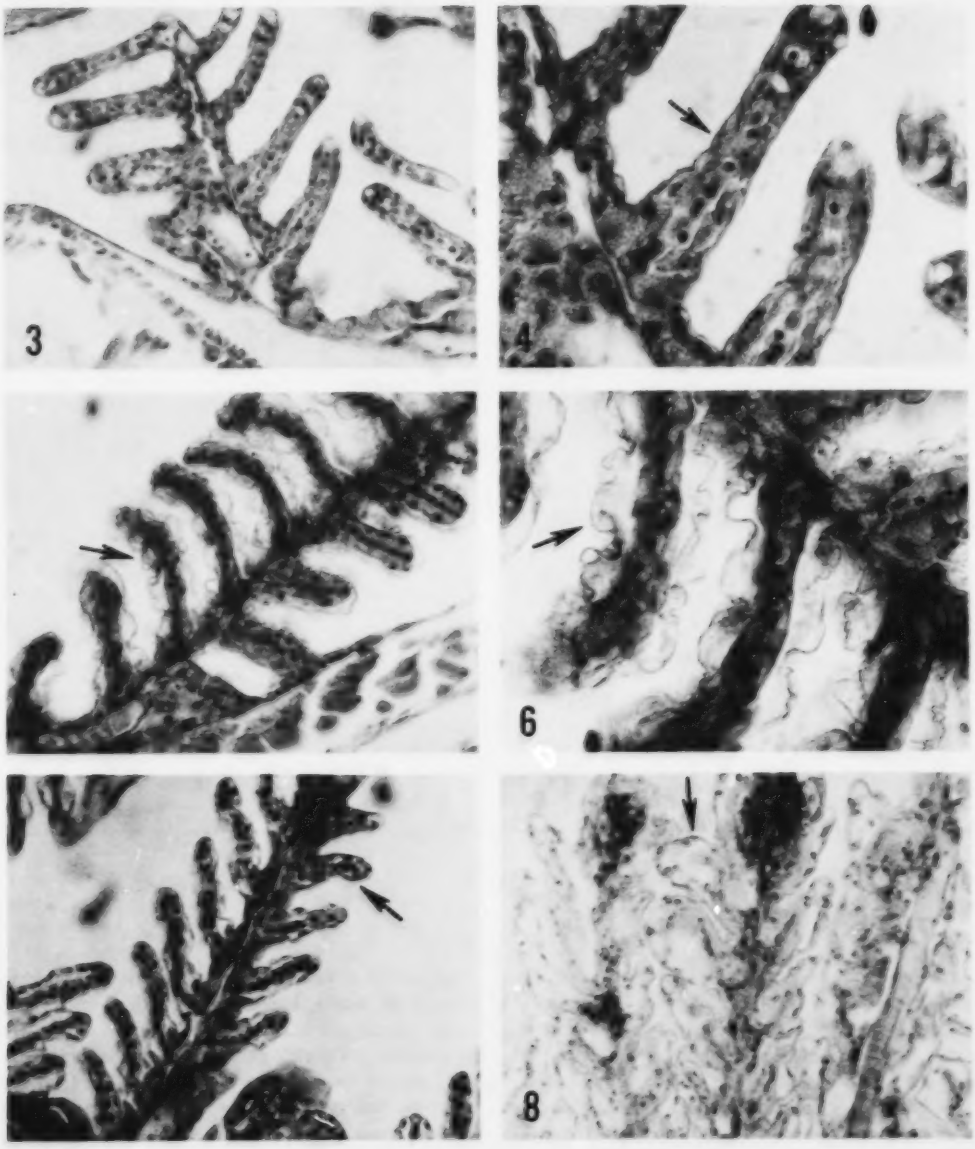
Concurrent exposure of spot to 0.05–0.07 mg l^{-1} TRC with a thermal shock also had little effect on survival. The only significant ($P < 0.05$) reduction in the number of survivors was observed for fish exposed at 28°C for 60 min.

The combined effect of TRC and thermal stress was most evident at a measured TRC range of 0.34–0.52 mg l^{-1}. In these tests, there was a consistent trend of increased sensitivity as temperature and exposure time increased (Fig. 9). Significant decreases in the number of survivors were observed for the following groups of fish: 20°C, 60 min exposure; 25°C, 30 and 60 min exposure; and 28°C, 30 and 60 min exposure.

DISCUSSION

The spot, *Leiostomus xanthurus*, has been shown to be sensitive both to chlorinated sewage effluents and to residual chlorine in sea-water (Virginia State Water Control Board, 1974). Massive fish kills, estimated at 5–10 million individuals, were observed in the Chesapeake Bay (James River) adjacent to two sewage treatment plants during May and June 1973.

In addition to spot, bluefish, *Pomatomus saltatrix*; white perch, *Morone americana*; grey sea trout,



Figs. 3 and 4. The pseudobranch epithelium consists of plump, eosinophilic cells surrounding lamellar capillaries.

Figs. 5 and 6. Pseudobranch alteration observed in juvenile spot exposed to 1.57 mg l^{-1} TRC for 95 min and sacrificed alive. Lamellar epithelial membranes are distorted and separated from other pseudobranch tissues.

Fig. 7. Gill filaments and lamellae from a juvenile spot (control).

Fig. 8. Gill filaments and lamellae from a juvenile spot exposed to 1.57 mg l^{-1} TRC and sacrificed alive. Filaments and lamellar organization are distorted by severe edema and separation of respiratory epithelium.

Table 2. Summary data for avoidance tests conducted with juvenile spot. χ^2 values are given for replicate tests at each exposure concentration of total residual chlorine (TRC). Significantly different values are indicated by asterisks

Test Temp °C	Replicate Test	TRC mg l^{-1}	Sum of df	Sum of χ^2	Pooled df	Pooled χ^2	Heterogeneity df	Heterogeneity Sum χ^2-Pooled χ^2
10	A	Control	5	1.00	1	0.80	4	0.20
		0.05	5	0.75	1	0.05	4	0.70
	B	Control	5	1.00	1	0.20	4	0.80
		0.05	5	1.25	1	0.80	4	0.45
	A	Control	5	1.25	1	0.80	4	0.45
		0.12	5	6.25	1	4.05*	4	2.20
	B	Control	5	1.00	1	0.45	4	0.55
		0.12	5	3.00	1	1.25	4	1.75
	A	Control	5	0.75	1	0.05	4	0.70
		0.18	5	13.00*	1	12.80‡	4	0.20
	B	Control	5	1.25	1	1.25	4	0.00
		0.18	5	15.25*	1	14.45‡	4	0.80
15	A	Control	5	1.00	1	0.80	4	0.20
		0.05	5	24.50‡	1	24.20‡	4	0.30
	B	Control	5	1.00	1	0.80	4	0.20
		0.05	5	16.75†	1	14.45†	4	2.30
	A	Control	5	0.75	1	0.20	4	0.55
		0.11	5	22.75‡	1	18.10‡	4	4.65
	B	Control	5	0.75	1	0.45	4	0.30
		0.11	5	19.75‡	1	20.00‡	4	0.25
	A	Control	5	1.00	1	0.80	4	0.20
		0.18	5	44.00‡	1	39.20‡	4	4.80
	B	Control	5	1.00	1	0.00	4	1.00
		0.18	5	18.25‡	1	16.20‡	4	2.05
20	A	Control	5	0.50	1	0.20	4	0.30
		0.05	5	17.75‡	1	14.50‡	4	3.25
	B	Control	5	0.75	1	0.05	4	0.70
		0.05	5	17.50‡	1	16.20‡	4	1.30
	A	Control	5	1.00	1	0.80	4	0.20
		0.13	5	13.50*	1	9.80‡	4	4.70
	B	Control	5	0.75	1	0.45	4	0.30
		0.13	5	18.75‡	1	18.10‡	4	0.65
	A	Control	5	0.75	1	0.45	4	0.30
		0.21	5	53.00‡	1	51.20‡	4	1.80
	B	Control	5	1.00	1	0.20	4	0.80
		0.21	5	38.75‡	1	36.50‡	4	2.25

* $P < 0.05$.
† $P < 0.01$.
‡ $P < 0.005$.

Cynoscion regalis; and menhaden, *Brevoortia tyrannus* were killed. Total residual chlorine concentrations ranging from 0.07–0.28 mg l^{-1} were measured in the James River where fish were dying.

Toxicity studies conducted by the Virginia Institute of Marine Science for the Virginia State Water Control Board confirmed that total residual chlorine in sea-water was toxic to juvenile spot. The estimated 96-h LC_{50} was 0.09 mg l^{-1} TRC. This value lies between our estimated incipient LC_{50} of 0.12 mg l^{-1} for flow-through bioassays conducted at 10°C and 0.06 mg l^{-1} TRC for bioassays at 15°C.

Histological examination of juvenile spot exposed to TRC in the incipient LC_{50} bioassay at 15°C revealed distortion and separation of lamellar epithelial membranes in the pseudobranch and gills. Although no consistent pseudobranch or gill alteration was found for the lower exposure concentrations of TRC, increased ventilation rates and production of copious quantities of mucus suggest that transport or diffusion of gases may have been inhibited, thus causing hypoxia and death.

Gill tissues are known to be subject to many artifactual changes during fixation and processing. However, alterations observed at 1.57 mg l^{-1} TRC in our study are reported here after careful comparison and contrasting with known artifacts, such as separation of epithelial layers due to osmotic pressure differences related to fixative penetration. These changes appear to be real effects that resulted from test exposure, not false effects of fixation and processing.

Servizi & Martens (1974), working in freshwater, have also observed necrosis and separation of epithelium from pillar cells in the gills of fingerling sockeye salmon, *Onchorhynchus nerka*, exposed to chlorinated sewage effluents.

We conducted avoidance tests to determine if juvenile spot would avoid measured TRC concentrations similar to those extimated to be incipient LC_{50} concentrations at 10 and 15°C. Tests at 10°C

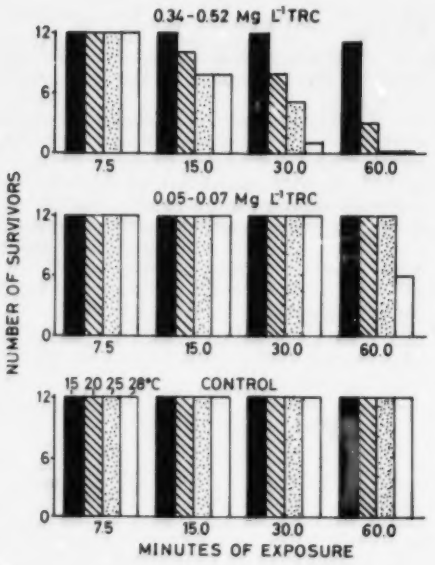


Fig. 9. Results of temperature stress–chlorine interaction tesis. Observations for the number of survivors were made 24 h after exposure to each test regime.

showed that spot would not consistently avoid a concentration of TRC as high as 0.12 mg l^{-1}. This concentration of TRC was estimated to be the incipient LC_{50} at 10°C. A TRC concentration of 0.18 mg l^{-1} was avoided by spot tested at 10°C. In the incipient LC_{50} bioassay, exposure to 0.18 mg l^{-1} caused 50% mortality after approximately 650 min of exposure.

Avoidance responses in tests at 15 and 20°C were observed for TRC concentrations as low as 0.05 mg l^{-1}, which is slightly below the estimated incipient LC_{50} of 0.06 mg l^{-1} at 15°C.

Meldrim *et al.* (1974) found that several estuarine fishes had a temperature-dependent avoidance response to chlorine. At 5°C, the silverside, *Menidia menidia*, avoided an average free-chlorine concentration of 0.10 mg l^{-1}. At 22°C, silversides avoided levels as low as 0.04 mg l^{-1} free chlorine. Field observations by Tsai (1968, 1970, 1973) suggest an avoidance response by fish exposed to chlorinated sewage effluents. A 'blocking effect' was observed in the upstream migration of certain anadromous and semi-anadromous fish trying to move past the chlorinated discharges from municipal sewage treatment plants.

The TRC-temperature interaction tests with juvenile spot demonstrated a significant increase in sensitivity to TRC as temperature and exposure time increased. Stober & Hanson (1974) found that pink salmon, *Onchorhynchus gorbuscha*, and chinook salmon, *O. tshawytscha*, also were more sensitive to residual chlorine at elevated temperatures. The greatest toxic effect was observed for a temperature increase of 9.9–10°C above acclimation and 0.5 mg l^{-1} residual chlorine. The LT_{50} (lethal time for 50% mortality) ranged from approximately 10 min for chinooks to 80 min for pink salmon. Hoss *et al.* (1975) in a similar study showed an increase in mortality of larval flounder, *Paralichthys* sp., occurred when they were exposed to combinations of thermal stress and chlorine in sea-water. For example, exposure of flounder to 0.5 mg l^{-1} free chlorine at the acclimation temperature did not casue any mortality; however, exposure at a temperature 10°C above the acclimation temperature caused 100% mortality.

Our study has shown that juvenile spot are sensitive to TRC in sea-water. Although spot may avoid TRC concentrations that would be lethal, provided they have an option for avoidance, elimination of habitat or a 'blocking effect' may occur in estuaries and adjacent river systems where chlorinated wastes are discharged.

Entrainment of juvenile spot (and other fishes and invertebrates) in coastal power plants that use chlorination to prevent biofouling could be particularly detrimental. This is indicated by our temperature stress-chlorine exposure tests.

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